

SEROLOGY

A. **DON'T'S** in submission of serum samples and tissue specimens. Experience in the past has shown the following problems associated with shipping serum samples or tissue specimens to the laboratory (in order of decreasing occurrence):

1. Bacterial contamination.
2. Serum not separated from clots.
3. Chemical (detergent, disinfectants, etc.) contamination of serum causing toxicity to tissue culture or otherwise adversely affecting desired tests.
4. Hemolysis.
5. Insufficient quantity.
6. Overheating or freezing of blood before serum extraction.
7. Leaky stoppers.
8. Broken containers.
9. Improper labeling.
10. Rotten tissue specimens.

B. **DO'S** in submission of serum samples and tissue specimens:

The following requirements will help prevent the above listed problems from occurring.

1. Serum
 - a) Collect blood aseptically into a sterile dry tube, refrigerate immediately, allow to clot, centrifuge, and transfer serum aseptically into a second tube.
 - b) Serum must be fresh, clear, unhemolyzed, and uncontaminated.
 - c) Red top Vacutainers[®], B-D, or other non-EDTA/heparin sterile commercially available tubes are ideal.
2. Label each tube (not stopper) with **tube number** and **vet code**. Be careful that writing will be legible upon arrival (use an indelible marker). Identify specimen in a way that will avoid confusion when results are reported. Avoid using animal names. Keep your own log of sample numbers to avoid duplication and confusion. Put small tubes in a box or tape to large cardboard so they will not be lost in the packing.
3. Serum is preferred to be separated, by centrifugation, from clotted blood prior to submission for testing. Do not let whole blood freeze or expose to direct sunlight or high temperature before decanting serum.
4. Submit at least 1.0 ml of serum for each test requested. Refrigerate the serum until shipment.
5. Tissues should be collected aseptically and placed individually in well-labeled whirl-pak bags. Place individual whirl-pak bag into a single air tight container and refrigerate immediately.
6. Swab samples should be kept in appropriate transport media and shipped in an insulated container with ice packs.
7. Refrigerated samples should be shipped with sufficient cool-pak bags and padding. Ship samples in a leak proof insulated container or in sturdy mailing carton which complies with postal or commercial carrier specifications (Page 10). Submit samples early in the week to avoid holding over the weekend by the carrier.
8. A complete and legible form(s), (including a complete history), must accompany all serologic submissions. Be certain to indicate vaccination history. "All and Current" is not an appropriate vaccination history.
9. Diagnostic serology samples must be submitted independently from program serology.
EXAMPLE: Serum for Lepto, IBR, BVD testing must be submitted separately from serum for "Bangs" serology.
10. All regulatory charts must include the submitting veterinarian's signature, vet code, and animal identification.
11. Flock check serology should be based upon a sample size of 20-30 samples per flock. National Poultry Improve Plan (NPIP) testing may require larger number of samples.

12. This laboratory is officially certified to perform complement fixation test for anaplasmosis, agar gel immunodiffusion test for bluetongue, bovine leukosis, and equine infectious anemia (the Coggins test for EIA), pseudorabies serology, and hog cholera fluorescent antibody testing.
13. Equine Infectious Anemia serology (Coggins test) requires at least two (2) ml of clear, fresh, unhemolyzed serum and takes up to 24 hours to complete. Each tube of serum submitted should be identified with **vet code** and the **tube number** corresponding to the tube number on the submission form. A **mane tag** is to be affixed to each horse tested and **tag number** recorded on the submission form. EIA results will be available after 8:30 A.M. the work day following the receiving of serum sample. If additional tests other than EIA are requested, please, submit two samples. The submission form must be completely and correctly completed and signed by the submitting veterinarian. **The lab cannot make any changes or fill in any missing information. All forms not properly completed will be returned for completion.**
14. **Exports:** The submitting veterinarian is responsible for informing the laboratory of any special requirements (i.e., dilutions, type of test). If this information is not supplied, a delay on the required test result could happen.
For information on test regulations, call:
Federal - USDA, APHIS, VS, Veterinarian in Charge - (615)781-5310
State - Office of the State Veterinarian - (615) 837-5120
The samples must be received 10 to 14 working days before the animals are scheduled to leave for the destination.
15. When calling for results, the laboratory must have the submitting veterinarian's name, the owner's name, the animal's name or ID, the sample number or ID, submitting date and bleeding date exactly as provided on the submission form.
16. Advance notice (7-10 days ahead) should be given (and will be greatly appreciated) when submitting a large number of samples.
17. For serodiagnosis in the individual animal, paired sera are recommended to test for specific antibody: the first sample taken when the animal is initially examined (acute phase serum) and the second sample 2-4 weeks later (convalescent phase serum). A rise in antibody titer between the paired samples is a basis for a specific serologic diagnosis for a particular disease. The paired serum samples should be submitted together in order to obtain a better understanding of the diagnostic significance of titers as they relate to the clinic status of the animal.
18. If only a single serum sample can be obtained, it must be taken from a convalescent animal. An acutely ill animal is virtually devoid of antibody against the particular disease-causing agent.
19. If only acutely ill animals are present, swabs or tissues for isolation or culture of the causative agent should also be submitted.

Key to abbreviations for Serology Test Chart:

AGID - Agar gel Immunodiffusion
CA - Card Agglutination
CF - Complement Fixation
ELISA - Enzyme Linked Immunosorbent Assay
FA - Fluorescent Antibody (Direct)
HI - Hemagglutination Inhibition
IFA - Indirect Fluorescent Antibody
IHA - Indirect Hemagglutination
LA - Latex Agglutination
NVSL - National Veterinary Services Laboratory
PCR - Polymerase Chain Reaction
TA - Tube Agglutination

SEROLOGY TEST CHART

| TEST | SPECIMEN | SHIPPING CONDITION | TYPE OF TEST | DAYS SET UP | LAB TIME | SPECIAL COMMENTS |
|--------------------------|---|--------------------|--------------|---------------------------|-------------|--|
| <u>Avian</u> | | | | | | |
| Avian Influenza | Serum | Refrigerated | AGID | M,Tu,W,F | 48 hours | Requires at least 1 ml of serum |
| Chlamydia | Spleen, liver, lung, air sac, conjunctival swab | Refrigerated | FA | M-F | 2 hours | |
| Mycoplasma gallisepticum | Serum | Refrigerated | ELISA, HI | Tu, Th | 48 hours | Requires at least 1 ml of serum. |
| | Trachea swab | Refrigerated | PCR | Special request required. | 36-48 hours | Submit in appropriate transport media. |
| Mycoplasma synoviae | Serum | Refrigerated | ELISA, HI | Tu, Th | 48 hours | Requires at least 1 ml of serum |
| | Trachea Swab | Refrigerated | PCR | Special request required. | 36-48 hours | Submit in appropriate transport media. |
| <u>Canine</u> | | | | | | |
| Anti-nuclear Antibody | Serum | Refrigerated | IFA | M-F | 3 hours | IgG, IgM determination. Samples run at 1:10, 1:50, 1:250 dilutions. Same day result if sample is received by noon. |
| Blastomycosis | Serum | Refrigerated | AGID | M-F | 24 hours | |
| Brucella canis | Serum | Refrigerated | CA | M-F | 30 mins. | Samples are screened by the card test. Positives will not be reported until confirmed by the tube test. |
| | | Refrigerated | TA | M,Tu,W,F | 48 hours | Samples run at 1:50, 1:100, 1:200 dilutions. Titers: 1:50 - Suspicious ≥1:100 - Positive |
| Calicivirus | Serum | Refrigerated | IFA | M-F | 3 hours | IgG, IgM determination. Samples run at 1:10, 1:50, 1:250 dilutions. Same day result if sample is received by noon. |
| Coronavirus | Small intestine | Refrigerated | FA | M-F | 2 hours | Same day result if sample is received by noon. |
| | Serum | Refrigerated | IFA | M-F | 3 hours | IgG, IgM determination. Samples run at 1:10, 1:50, 1:250 dilutions. Same day result if sample is received by noon. |
| Cryptococcus | Serum | Refrigerated | LA | M-F | 30 mins. | |

| TEST | SPECIMEN | SHIPPING CONDITION | TYPE OF TEST | DAYS SET UP | LAB TIME | SPECIAL COMMENTS |
|--------------------------------|---|--------------------|--------------|-------------|----------|--|
| Distemper Virus | Lung, kidney, spleen, urinary bladder, brain, stomach, liver, blood smear | Refrigerated | FA | M-F | 2 hours | Same day result if sample is received by noon. |
| | Serum | Refrigerated | IFA | M-F | 3 hours | IgG, IgM determination. Samples run at 1:10, 1:50, 1:250 dilutions. Same day result if sample is received by noon. |
| Ehrlichia canis | Serum | Refrigerated | IFA | M-F | 3 hours | Sample is tested at 1:20 dilution. Same day result if sample is received by noon. |
| Herpesvirus | Lung, liver, kidney, spleen, lymph node | Refrigerated | FA | M-F | 2 hours | Same day result if sample is received by noon. |
| | Serum | Refrigerated | IFA | M-F | 3 hours | IgG, IgM determination. Samples run at 1:10, 1:50, 1:250 dilutions. Same day result if sample is received by noon. |
| Histoplasmosis | Serum | Refrigerated | AGID | M-F | 24 hours | |
| Infectious Canine Hepatitis | Serum | Refrigerated | IFA | M-F | 3 hours | IgG, IgM determination. Samples run at 1:10, 1:50, 1:250 dilutions. Same day result if sample is received by noon. |
| Neospora caninum | Brain, lung, liver, kidney, muscle, skin | Refrigerated | FA | M-F | 2 hours | Same day result if sample is received by noon. |
| | Serum | Refrigerated | IFA | M-F | 3 hours | Tested at 1:25. Same day result if sample is received by noon. |
| Parvovirus | Intestine (jejunum, ileum), spleen, mesenteric lymph node | Refrigerated | FA | M-F | 2 hours | Same day result if sample is received by noon. |
| | Serum | Refrigerated | IFA | M-F | 3 hours | IgG, IgM determination. Samples run at 1:10, 1:50, 1:250 dilutions. Same day result if sample is received by noon. |
| <u>Equine</u> | | | | | | |
| Equine Infectious Anemia (EIA) | Serum | Refrigerated | AGID | M-F | 24 hours | Complete EIA form (VS Form 10-11) with submitting veterinarian's signature is required. |

| TEST | SPECIMEN | SHIPPING CONDITION | TYPE OF TEST | DAYS SET UP | LAB TIME | SPECIAL COMMENTS |
|-------------------------------------|--|--------------------|--------------|-------------|----------|--|
| Equine Influenza | Lung, nasal swab | Refrigerated | FA | M-F | 2 hours | Same day result if sample is received by noon. |
| | Serum | Refrigerated | IFA | M-F | 3 hours | Samples run at 1:10, 1:50, 1:250 dilutions. Same day result if sample is received by noon. |
| Equine Rhinopneumonitis Virus (ERV) | Lung, liver, spleen, fetal tissues | Refrigerated | FA | M-F | 2 hours | Same day result if sample is received by noon. |
| | Serum | Refrigerated | SN | Tu,W,Th,F | 4-5 days | Titers begin at 1:4. |
| Potomac Horse Fever | Serum | Refrigerated | IFA | M-F | 3 hours | Samples run at 1:20, 1:40, 1:80, 1:160 dilutions. Same day result if sample is received by noon. |
| <u>Feline</u> | | | | | | |
| Chlamydia | Conjunctival smear, nasal swab, lung | Refrigerated | FA | M-F | 2 hours | Same day result if sample is received by noon. |
| Feline Calicivirus | Serum | Refrigerated | IFA | M-F | 3 hours | Samples are tested for IgG titers at 1:10, 1:50, 1:250 dilutions. Same day result if sample is received by noon. |
| Feline Herpesvirus | Nasal swab, conjunctival swab, tonsil, trachea, lung | Refrigerated | FA | M-F | 2 hours | Same day result if sample is received by noon. |
| | Serum | Refrigerated | IFA | M-F | 3 hours | Sample are tested for IgG titers at 1:10, 1:50, 1:250 dilutions. Same day result if sample is received by noon. |
| Feline Immunodeficiency Virus (FIV) | Serum | Refrigerated | IFA | M-F | 3 hours | Sample is tested at 1:25 dilution. |
| Feline Infectious Peritonitis (FIP) | Affected tissues (liver, spleen, lymph nodes) | Refrigerated | FA | M-F | 2 hours | Same day result if sample is received by noon. |
| | Serum, pleural or peritoneal fluid | Refrigerated | IFA | M-F | 3 hours | Sample is tested at 1:5000 dilution. Same day result if sample is received by noon. |

| TEST | SPECIMEN | SHIPPING CONDITION | TYPE OF TEST | DAYS SET UP | LAB TIME | SPECIAL COMMENTS |
|--|--|--------------------|--------------|-------------|----------|--|
| Feline Leukemia | Blood smears | Slide mailers | IFA | M-F | 3 hours | Submit at least 2 smears. Thick smears are not acceptable. Do not use old or used slides. Same day result if sample is received by noon. |
| | Serum | Refrigerated | IFA | M-F | 3 hours | Sample is tested at 1:25 dilution. |
| Panleukopenia | Small intestine, lymph node, spleen, aborted fetus | Refrigerated | FA | M-F | 2 hours | Same day result if sample is received by noon. |
| | Serum | Refrigerated | IFA | M-F | 3 hours | Samples are tested for IgG titers at 1:10, 1:50, 1:250 dilutions. Same day result if sample is received by noon. |
| Toxoplasmosis | Serum | Refrigerated | IHA | M-F | 3 hours | Positive titer \geq 1:64. Same day result if sample is received by noon. |
| <u>Porcine</u> | | | | | | |
| E.coli Pilus | Small intestine, feces | Refrigerated | IFA | M-F | 3 hours | Test for K88, K99, P987 serotypes. Same day result if sample is received by noon. |
| Hemagglutinating Encephalomyelitis Virus | Brain | Refrigerated | FA | M-F | 2 hours | Same day result if sample is received by noon. |
| Leptospirosis | Serum | Refrigerated | MA | M-F | 4 hours | Test for 6 serovars - canicola, grippityphosa, hardjo, icterohemorrhagiae, pomona, and bratislava. Samples tested at 1:100, 1:200, 1:400, 1:800 dilutions. |
| | Kidney | Refrigerated | FA | M-F | 2 hours | Same day result if sample is received by noon. |
| Porcine Parvovirus | Fetal tissues | Refrigerated | FA | M-F | 2 hours | Same day result if sample is received by noon. |
| | Fetal serum or fetal fluid | Refrigerated | IFA | M-F | 3 hours | Samples run at 1:10, 1:50, 1:250 dilutions. A single serum sample from the dam is of little value since the breeding herd is often seropositive. |
| Porcine Respiratory and Reproductive Syndrome (PRRS) | Serum | Refrigerated | IFA | M-F | 3 hours | Sample is tested at 1:20 dilution. |

| TEST | SPECIMEN | SHIPPING CONDITION | TYPE OF TEST | DAYS SET UP | LAB TIME | SPECIAL COMMENTS |
|---|---|--------------------|--------------|--------------------------|----------|--|
| Pseudorabies | Brain, lung, tonsil, kidney | Refrigerated | FA | M-F | 2 hours | Same day result if sample is received by noon. |
| | Serum | Refrigerated | SN | M,Tu,W,F | 48 hours | Samples must be clear, unhemolyzed serum that is poured off clot. |
| | Serum | Refrigerated | LA | Special request required | 1 hour | Results are reported as positive or negative. |
| Rotavirus | Feces, intestine | Refrigerated | ELISA | M-F | 3 hours | Same day result if sample is received by noon. |
| Swine Influenza | Nasal secretions, trachea, lung | Refrigerated | FA | M-F | 2 hours | |
| | Serum | Refrigerated | IFA | M-F | 3 hours | Samples are tested for IgG titers at 1:10, 1:50, 1:250 dilutions. |
| Transmissible Gastroenteritis (TGE) | Intestine (jejunum, ileum) | Refrigerated | FA | M-F | 2 hours | Same day result if sample is received by noon. |
| | Serum | Refrigerated | SN | M-F | 6 days | Titers begin at 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256 |
| <u>Ruminant</u> | | | | | | |
| Anaplasmosis | Serum | Refrigerated | CA | M-F | 30 min. | Samples must be at least 48 hours old from time of collection to time test conducted. Time of collection must be written on submission form. Result is reported as positive or negative. |
| | Serum | Refrigerated | CF | Tuesday | 24 hours | For export purposes. Advance notification (at least 1 week ahead) is required. Titers begin at 1:5. |
| Bluetongue | Serum | Refrigerated | AGID | M-F | 24 hours | |
| Bovine Leukosis (BLV) | Serum | Refrigerated | AGID | M,Tu,W,F | 48 hours | |
| Bovine Respiratory Syncytial Virus (BRSV) | Lung, bronchial lymph node | Refrigerated | FA | M-F | 2 hours | Same day result if sample is received by noon. |
| | Serum | Refrigerated | IFA | M-F | 3 hours | Samples run at 1:10 dilution. Same day result if sample is received by noon. |
| Bovine Viral Diarrhea (BVD) | Lung, intestine, turbinate, trachea, swabs from lesions, fetal organs | Refrigerated | FA | M-F | 2 hours | Same day result if sample is received by noon. |
| | Serum | Refrigerated | SN | Tu,W,Th,F | 6 days | Titers: 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256 |

| TEST | SPECIMEN | SHIPPING CONDITION | TYPE OF TEST | DAYS SET UP | LAB TIME | SPECIAL COMMENTS |
|--|---|--------------------|--------------|-------------|----------|---|
| Chlamydia | Lymph node, tissues of aborted fetus, joint fluid | Refrigerated | FA | M-F | 2 hours | Specifically request chlamydia. |
| Clostridium | Gangrenous muscle, liver, lung | Refrigerated | FA | M-F | 2 hours | Test for C. chauvoei, novyi, and septicum. Same day result if sample is received by noon. |
| Coronavirus | Intestine (spiral colon, ileum, and jejunum) | Refrigerated | FA | M-F | 2 hours | Same day result if sample is received by noon. |
| E.coli Pilus | Small intestine | Refrigerated | IFA | M-F | 3 hours | Test for K99 serotype. Same day result if sample is received by noon. |
| Epizootic Hemorrhagic Disease (EHD) | Serum | Refrigerated | AGID | M-F | 24 hours | |
| Infectious Bovine Rhinotracheitis (IBR) | Lung, trachea, turbinate, aborted fetal tissues | Refrigerated | FA | M-F | 2 hours | Same day result if sample is received by noon.` |
| | Serum | Refrigerated | SN | Tu,W,Th,F | 6 days | Titers: 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256 |
| Johne's Disease (Mycobacterium paratuberculosis) | Serum | Refrigerated | CF | Thursday | 24 hours | Titers: 1:8 - Negative 1:16 - Suspicious 1:32 - Positive |
| Leptospirosis | Kidney | Refrigerated | FA | M-F | 2 hours | Same day result if sample is received by noon. |
| | Serum | Refrigerated | MA | M-F | 4 hours | Test for 6 serovars - canicola, grippotyphosa, hardjo, icterohemorrhagiae, pomona, and bratislava. Samples tested at 1:100, 1:200, 1:400, 1:800 |
| Listeria | Serum | Refrigerated | CA | M-F | 30 min. | Test for Type 1 and Type 4 serotypes. Screened at 1:20. Titers: 1:20, 1:40, 1:80, 1:160 |
| Neospora caninum | Brain, lung, kidney, liver | Refrigerated | FA | M-F | 2 hours | Same day result if sample is received by noon. |
| | Serum | Refrigerated | IFA | M-F | 3 hours | Serum run at 1:400 dilution. Same day result if sample is received by noon. |
| Rotavirus | Feces, intestine (spiral colon, ileum, and jejunum) | Refrigerated | ELISA | M-F | 3 hours | Same day result if sample is received by noon. |